

Off-line comprehensive IP-RPLC x CE and IEX-LC x CE for oligonucleotide impurity mapping

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The increasing development of oligonucleotide (ON) based pharmaceuticals necessitates the design of new separation protocols allowing impurity determinations next to the active pharmaceutical ingredient. Because of the rapidly increasing number of structural and chemical variations in ONs up to 30 bases long, conventional 1-D based separations are insufficient for adequate impurity mapping. Capillary gel electrophoresis (CE) has demonstrated to be a reproducible technique capable to separate (n-1) ONs fragments involving predominately a separation by size mechanism. Alternatively HPLC allows the separation of ONs not only based on their size but also on their nucleotide composition. Ion-pairing reverse phase liquid chromatography (IP-RPLC) and ion exchange liquid chromatography (IEX-LC) have been widely used for the analysis of ONs < 20 *mer*. Nevertheless, a general limitation in both LC techniques is the loss in resolution as the ONs length increments. By the combination of LC and CE an orthogonal methodology capable to differentiate ONs fragments by size and by nucleotide composition can be obtained.

Therefore offline comprehensive IP-RPLC x CE and IEX-LC x CE methodologies for the separation of adenosine thymidine, cytosine and uracil ONs (up to 35 *mer*) were developed. IP-RPLC using a C18 column and IEX-LC in a strong ion exchanger polymer column were used as first dimensions. CE in entangled polymer solution was implemented as second dimension. A complete separation of the ONs mixture was accomplished with a theoretical peak capacity of 6435 and 6993 for IP-RPLC x CE and IEX-LC x CE, respectively. This methodology shows promising results for its implementation in the analysis of complex biological matrixes involving the presence of therapeutic ONs and double and single DNA strands.